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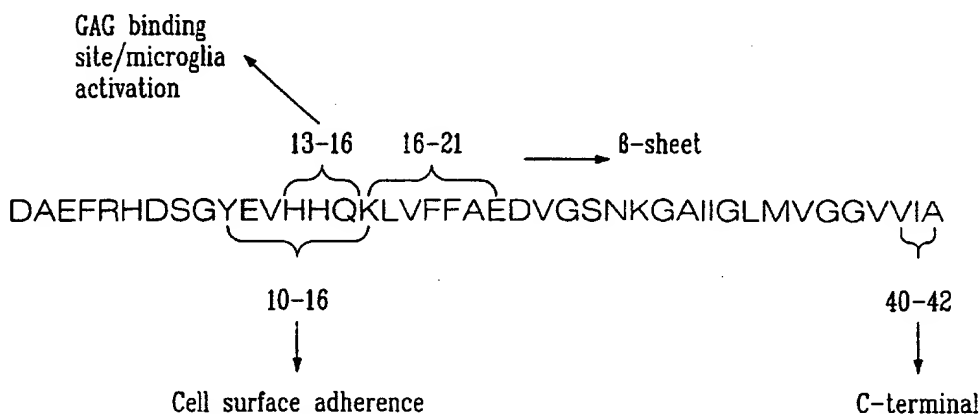
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(54) Title: **VACCINE FOR THE PREVENTION AND TREATMENT OF ALZHEIMER'S AND AMYLOID RELATED DISEASES**



(57) Abstract: The present invention relates to a stereochemically based "non-self" antigen vaccine for the prevention and/or treatment of Alzheimer's and other amyloid related diseases. The present invention provides a vaccine for the prevention and treatment of Alzheimer's and other amyloid related diseases, which overcomes the drawbacks associated with using naturally occurring peptides, proteins or immunogens.

VACCINE FOR THE PREVENTION AND TREATMENT OF ALZHEIMER'S AND AMYLOID RELATED DISEASES

RELATED APPLICATIONS

- 5 This application claims the benefit of priority under 35 U.S.C. 119(e) to copending U.S. Provisional Application No. 60/168,594, filed on November 29, 1999, and under 35 U.S.C. 120 of copending U.S. application No. _____, filed on November 28, 2000, the entire contents of both applications are incorporated herein by reference.

BACKGROUND OF THE INVENTION

- 10 The present invention relates to a new stereochemically based "non-self" antigen vaccine for the prevention and/or treatment of Alzheimer's and other amyloid related diseases.

- Amyloidosis refers to a pathological condition characterized by the presence of amyloid fibers. Amyloid is a generic term referring to a group of diverse but specific protein
15 deposits (intracellular and/or extracellular) which are seen in a number of different diseases. Though diverse in their occurrence, all amyloid deposits have common morphologic properties, stain with specific dyes (e.g., Congo red), and have a characteristic red-green birefringent appearance in polarized light after staining. They also share common ultrastructural features and common x-ray diffraction and infrared spectra.

- 20 Amyloid-related diseases can either be restricted to one organ or spread to several organs. The first instance is referred to as "localized amyloidosis" while the second is referred to as "systemic amyloidosis".

- Some amyloidotic diseases can be idiopathic, but most of these diseases appear as a complication of a previously existing disorder. For example, primary amyloidosis can appear
25 without any other pathology or can follow plasma cell dyscrasia or multiple myeloma. Secondary amyloidosis is usually seen associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis). A familial form of secondary amyloidosis is also seen in Familial Mediterranean Fever (FMF). This familial type of amyloidosis, as one of the other types of familial amyloidosis, is genetically inherited and is
30 found in specific population groups. In these two types of amyloidosis, deposits are found in several organs and are thus considered systemic amyloid diseases. Another type of systemic amyloidosis is found in long-term hemodialysis patients. In each of these cases, a different amyloidogenic protein is involved in amyloid deposition.

"Localized amyloidoses" are those that tend to involve a single organ system. Different amyloids are also characterized by the type of protein present in the deposit. For example, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease and the like are characterized by the appearance and accumulation of a protease-resistant form of a prion protein (referred to as A β Sc or PrP-27) in the central nervous system. Similarly, Alzheimer's disease, another neurodegenerative disorder, is characterized by neuritic plaques and neurofibrillary tangles. In this case, the plaque and blood vessel amyloid is formed by the deposition of fibrillar A β amyloid protein. Other diseases such as adult-onset diabetes (Type II diabetes) are characterized by the localized accumulation of amyloid in the pancreas.

Once these amyloids have formed, there is no known, widely accepted therapy or treatment which significantly dissolves the deposits *in situ*.

Each amyloidogenic protein has the ability to organize into β -sheets and to form insoluble fibrils which get deposited extracellularly or intracellularly. Each amyloidogenic protein, although different in amino acid sequence, has the same property of forming fibrils and binding to other elements such as proteoglycan, amyloid P and complement component. Moreover, each amyloidogenic protein has amino acid sequences which, although different, will show similarities such as regions with the ability to bind to the glycosaminoglycan (GAG) portion of proteoglycan (referred to as the GAG binding site) as well as other regions which will promote β -sheet formation.

In specific cases, amyloidotic fibrils, once deposited, can become toxic to the surrounding cells. As per example, the A β fibrils organized as senile plaques have been shown to be associated with dead neuronal cells and microgliosis in patients with Alzheimer's disease. When tested *in vitro*, A β peptide was shown to be capable of triggering an activation process of microglia (brain macrophages), which would explain the presence of microgliosis and brain inflammation found in the brain of patients with Alzheimer's disease.

In another type of amyloidosis seen in patients with Type II diabetes, the amyloidogenic protein IAPP has been shown to induce β -islet cell toxicity *in vitro*. Hence, appearance of IAPP fibrils in the pancreas of Type II diabetic patients could contribute to the loss of the β islet cells (Langerhans) and organ dysfunction.

People suffering from Alzheimer's disease develop a progressive dementia in adulthood, accompanied by three main structural changes in the brain: diffuse loss of neurons in multiple parts of the brain; accumulation of intracellular protein deposits termed neurofibrillary tangles; and accumulation of extracellular protein deposits termed amyloid or senile plaques, surrounded by misshapen nerve terminals (dystrophic neurites). A main

constituent of these amyloid plaques is the amyloid- β peptide ($A\beta$), a 40-42 amino-acid protein that is produced through cleavage of the β -amyloid precursor protein (APP). Although symptomatic treatments exist for Alzheimer's disease, this disease cannot be prevented nor cured at this time.

5 The use of a vaccine to treat Alzheimer's disease is possible in principle (Schenk, D. et al., (1999) Nature 400, 173-177). Schenk et al. show that, in a transgenic mouse model of brain amyloidosis (as seen in Alzheimer's disease), immunization with $A\beta$ peptide inhibits the formation of amyloid plaques and the associated dystrophic neurites. In that study, a vaccine using the human aggregated all-L peptide as immunogen prevented the formation of β -amyloid
10 plaque, astrogliosis and neuritic dystrophy in vaccinated transgenic mice.

However, it is apparent that there are a number of drawbacks to using an endogenous protein as a vaccine (or a protein naturally present in the animal being vaccinated). Some of these drawbacks include:

- 15 • Possible development of autoimmune disease due to the generation of antibodies against "self" protein.
- Difficulty in eliciting an immune response due to the failure of the host immune system to recognize "self" antigens.
- Possible development of an acute inflammatory response.

20 SUMMARY OF THE INVENTION

The present invention relates to a stereochemically based "non-self" antigen vaccine for the prevention and/or treatment of Alzheimer's and other amyloid related diseases. One aim of the present invention is to provide a vaccine for the prevention and treatment of Alzheimer's and other amyloid related diseases, which overcomes the drawbacks associated with using
25 naturally occurring peptides, proteins or immunogens.

In an embodiment, a vaccine is provided which is produced using a "non-self" peptide or protein synthesized from the unnatural D-configuration amino acids, to avoid the drawbacks of using "self" proteins. In accordance with the present invention, the peptides need not be aggregated to be operative or immunogenic as opposed to the prior art vaccines.

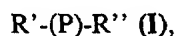
30 In another embodiment, there is provided a method for preventing and/or treating an amyloid-related disease in a subject, which features administering to the subject an antigenic amount of an all-D peptide which elicits production of antibodies against the all-D peptide, and elicit an immune response by the subject, therefore preventing fibrillogenesis and associated cellular toxicity, wherein the antibodies interact with at least one region of an amyloid protein,

e.g., β sheet region and GAG-binding site region, immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof. These vaccines may be used in the prevention and/or treatment of amyloid related diseases, and in the manufacture of medicaments for preventing and/or treating amyloid-related diseases.

In a further embodiment of the invention, a vaccine for preventing and/or treating an amyloid-related disease in a subject comprises an antibody which interacts with amyloid proteins to prevent fibrillogenesis, wherein the antibodies are raised against an antigenic amount of an all-D peptide interacting with at least one region of an amyloid protein, e.g., β sheet region and GAG-binding site region, $A\beta$ (1-42, all-D), immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof. These vaccines may be used in the prevention and/or treatment of amyloid related diseases, and in the manufacture of medicaments for preventing and/or treating amyloid-related diseases.

Still in a further embodiment, there is provided a method for preventing and/or treating an amyloid-related disease in a subject, which comprises administering to the subject an antigenic amount of an all-D peptide which interacts with at least one region of an amyloid protein, e.g., β sheet region and GAG-binding site region, $A\beta$ (1-42), immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof, wherein the compound elicits an immune response by the subject and therefore prevents fibrillogenesis.

In a preferred embodiment of the present invention, the compound is a compound of Formula I:



wherein

P is an all-D peptide interacting with at least one region of an amyloid protein, e.g., β sheet region and GAG-binding site region, $A\beta$ (1-42, all-D), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;

R' is an N-terminal substituent, e.g.:

- hydrogen;
- lower alkyl groups, e.g., acyclic or cyclic having 1 to 8 carbon atoms, without or with functional groups, e.g., carboxylate, sulfonate and phosphonate;

- aromatic groups;
- heterocyclic groups; and
- acyl groups, e.g., alkylcarbonyl, arylcarbonyl, sulfonyl and phosphonyl groups; and

5 R'' is a C-terminal substituent, e.g., hydroxy, alkoxy, aryloxy, unsubstituted or substituted amino groups.

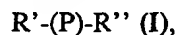
In an embodiment, R' and R'' are identical or different, wherein alkyl or aryl group of R' and R'' are further substituted with functionalities such as halide (e.g., F, Cl, Br, and I), hydroxyl, alkoxy, aryloxy, hydroxycarbonyl, alkoxy carbonyl, aryloxy carbonyl, carbamyl, 10 unsubstituted or substituted amino, sulfo or alkyloxysulfonyl, phosphono or alkoxyphosphonyl groups.

When the compound has an acid functional group, it can be in the form of a pharmaceutically acceptable salt or ester. When the compound has a basic functional group, it can be in the form of a pharmaceutically acceptable salt.

15 In a preferred embodiment of the present invention, the subject is a human being.

In yet another embodiment of the present invention, the amyloid related disease may be Alzheimer's disease.

In another embodiment of the present invention, there is provided a method for preventing and/or treating of an amyloid related disease in a subject, comprising administering 20 to the subject an antigenic amount of a compound of Formula I:



wherein

P is an all-D peptide interacting with at least one region of an amyloid protein, e.g., β sheet region and GAG-binding site region, A β (1-42, all-D), 25 immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;

R' is an N-terminal substituent selected from the group consisting of:

- hydrogen;
- 30 ▪ lower alkyl groups, e.g., acyclic or cyclic having 1 to 8 carbon atoms, without or with functional groups, e.g., carboxylate, sulfonate and phosphonate;
- aromatic groups;

- heterocyclic groups; and
- acyl groups, e.g., alkylcarbonyl, arylcarbonyl, sulfonyl and phosphonyl groups; and

5 R'' is a C-terminal substituent, e.g., hydroxy, alkoxy, aryloxy, unsubstituted or substituted amino groups.

In accordance with this method, the compound elicits an immune response by the subject, preventing fibrillogenesis.

10 In accordance with a preferred embodiment of the present invention, there is provided a vaccine for preventing and/or treating an amyloid-related disease in a subject, comprising an antigenic amount of an all-D peptide which interacts with at least one region of an amyloid protein, e.g., β sheet region and GAG-binding site region, A β (1-42, all-D) peptide, immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof, wherein the compound elicits an immune response by the subject and prevents fibrillogenesis.

15

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 illustrates the targeted sites for the antigenic fragments;

FIG. 2 illustrates the effect of 1 mg/ml of antibodies raised against D and L forms of A β (16-21) on fibrillogenesis;

20 FIG. 3 illustrates the effect of 0.5 mg/ml of antibodies raised against D and L forms of A β (16-21) on fibrillogenesis;

FIGs. 4A to 4C illustrate electron micrographs showing the effect of anti-D KLVFFA peptide antibodies (FIG. 4B) and anti-L KLVFFA peptide antibodies (FIG. 4C) with respect to a control (FIG. 4A) on fibrillogenesis;

25 FIGs. 5A to 5D illustrate the immunohistochemistry of anti-D KLVFFA on aggregated A β peptide in brain sections of retrosplenial cortex (FIG. 5A) and parietal cortex (FIG. 5C) and the histochemistry (Thioflavin S assay) of anti-D KLVFFA on aggregated A β peptide in the same brain sections of retrosplenial cortex (FIG. 5B) and parietal cortex (FIG. 5D);

30 FIGs. 6A to 6D illustrate the immunohistochemistry of anti-L KLVFFA antibodies on aggregated A β peptide in brain sections of parietal cortex (FIG. 6A) and entorhinal cortex (FIG. 6C) and the histochemistry (Thioflavin S assay) of anti-L KLVFFA antibodies on aggregated A β peptide in the same brain sections of parietal cortex (FIG. 6B) and entorhinal cortex (FIG. 6D); and

FIG. 7 illustrates the response of rabbits to KLH-conjugated all-L and all-D KLVFFA.

DETAILED DESCRIPTION OF THE INVENTION

5 For the purpose of the present disclosure, the following terms are defined below.

The term "peptidomimetic" includes non-peptide compounds which mimic the structural or the functional properties of a peptide.

The term "antigenic fragment thereof" includes fragments of peptides which are capable of eliciting an immune response in a subject.

10 The term "amyloid related diseases" includes diseases associated with the accumulation of amyloid which can either be restricted to one organ, "localized amyloidosis", or spread to several organs, "systemic amyloidosis". Secondary amyloidosis may be associated with chronic infection (such as tuberculosis) or chronic inflammation (such as rheumatoid arthritis), including a familial form of secondary amyloidosis which is also seen in Familial
15 Mediterranean Fever (FMF) and another type of systemic amyloidosis found in long-term hemodialysis patients. Localized forms of amyloidosis include, without limitation, diabetes type II and any related disorders thereof, neurodegenerative diseases such as scrapie, bovine spongiform encephalitis, Creutzfeldt-Jakob disease, Alzheimer's disease, Cerebral Amyloid Angiopathy, and prion protein related disorders.

20 Except as otherwise expressly defined herein, the abbreviations used herein for designating the amino acids and the protective groups are based on recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Biochemistry*, 1972, 11:1726-1732).

The A β (16-21) site is known to play an important role in initiating the harmful process of A β peptide amyloidogenesis. It is also known that when these peptides are made
25 from D-amino acids, they retain their ability to interact with the natural all-L-homologous sequence, thereby preventing amyloidogenesis.

Other amyloid proteins which may be used in the present invention include, without limitation, IAPP, β 2-microglobuline, amyloid A protein, and prion-related proteins.

The vaccine of the present invention, prepared from all-D-A β (16-21), D-A β (10-16),
30 D-A β (1-40), D-A β (1-42) or the C-terminal region of D-A β (1-42), is believed to elicit an immune response in the host or in producing antibodies that recognize the naturally occurring target. As used herein, "all-D" includes peptides having $\geq 75\%$, $\geq 80\%$, $\geq 85\%$, $\geq 90\%$, $\geq 95\%$, and 100% D-configuration amino acids. Also, the vaccine of the present invention does not present the drawbacks of using "self" proteins and does not need to be aggregated to induce an

immune response. For example, the antibodies raised against the all-D-A β (16-21) peptide can be expected to recognize the all-L-A β (16-21) peptide sequence.

The elicited antibodies present in the host having received the vaccine of the present invention bind at the A β (16-21) site or other sites such as the C-terminal region of A β and have the same or even greater ability to prevent amyloidogenesis as do the short peptides themselves. The vaccine of the present invention causes the generation of effective antiamyloidogenic antibodies in the vaccinated host.

A suggested immunization procedure is as follows:

- a) prepare a vaccine from an all-D peptide having a sequence substantially the same as that of a naturally occurring β amyloid peptide, namely A β (all-L). The all-D peptide includes a full length A β (1-42, all-D), a peptide derived from an immunogenic fragment of A β (1-42, all-D), and a related peptidomimetic;
- b) immunize a host with the vaccine to generate an antibody in the host with a binding site capable of preventing fibrillogenesis.

Suitable pharmaceutically acceptable carriers include, without limitation, any non-immunogenic pharmaceutical adjuvants suitable for oral, parenteral, intravascular (IV), intraarterial (IA), intramuscular (IM), and subcutaneous (SC) administration routes, such as phosphate buffer saline (PBS).

The pharmaceutical carriers may contain a vehicle, which carries antigens to antigen-presenting cells. Examples of vehicles are liposomes, immune-stimulating complexes, microfluidized squalene-in-water emulsions, microspheres which may be composed of poly(lactic/glycolic) acid (PLGA). Particulates of defined dimensions (<5 micron) include, without limitation, oil-in-water microemulsion (MF59) and polymeric microparticles.

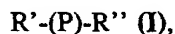
The carriers of the present invention may also include chemical and genetic adjuvants to augment immune responses or to increase the antigenicity of antigenic immunogens. These adjuvants exert their immunomodulatory properties through several mechanisms such as lymphoid cells recruitment, cytokine induction, and the facilitation of DNA entry into cells. Cytokine adjuvants include, without limitation, granulocyte-macrophage colony-stimulating factor, interleukin-12, GM-CSF, synthetic muramyl dipeptide analog or monophosphoryl lipid A. Other chemical adjuvants include, without limitation, lactic acid bacteria, Al(OH)₃, muramyl dipeptides and saponins.

The peptide may be coupled to a carrier that will modulate the half-life of the circulating peptide. This will allow the control on the period of protection. The peptide-carrier may also be emulsified in an adjuvant and administered by usual immunization route.

The vaccine of the present invention will, for the most part, be administered parenterally, such as intravascularly (IV), intraarterially (IA), intramuscularly (IM), subcutaneously (SC), or the like. In some instances, administration may be oral, nasal, rectal, transdermal or aerosol, where the nature of the vaccine allows for transfer to the vascular system. Usually a single injection will be employed although more than one injection may be used, if desired. The vaccine may be administered by any convenient means, including syringe, trocar, catheter, or the like. Preferably, the administration will be intravascularly, where the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow, e.g., intravenously, peripheral or central vein. Other routes may find use where the administration is coupled with slow release techniques or a protective matrix.

The use of the vaccine of the present invention in preventing and/or treating Alzheimer's disease and other amyloid related diseases can be validated by raising antibodies against the corresponding all-D peptide and testing them to see if they can effectively inhibit or prevent the fibrillogenesis of the natural amyloid peptide (all-L).

The compounds used to prepare vaccines in accordance with the present invention have the common structure of Formula I:



wherein

- P** is an all-D peptide interacting with at least one region of an amyloid protein, e.g., β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;
- R'** is an N-terminal substituent selected from the group consisting of:
- hydrogen;
 - lower alkyl groups, e.g., acyclic or cyclic having 1 to 8 carbon atoms, without or with functional groups, e.g., carboxylate, sulfonate and phosphonate;
 - aromatic groups;
 - heterocyclic groups; and
 - acyl groups, e.g., alkylcarbonyl, arylcarbonyl, sulfonyl and phosphonyl groups; and
- R''** is a C-terminal substituent, e.g., hydroxy, alkoxy, aryloxy, unsubstituted or substituted amino groups.

R' and R'' may be identical or different; the alkyl or aryl group of R' and R'' may further be substituted with organic functionalities selected from the group of halides (F, Cl, Br, and I), hydroxyl, alkoxyl, aryloxy, hydroxycarbonyl, alkoxycarbonyl, aryloxy, carbamyl, unsubstituted or substituted amino, sulfo or alkyloxysulfonyl, phosphono or alkoxyphosphonyl, and the like.

Where a functional group is an acid, its pharmaceutically acceptable salt or ester is in the scope of this invention. Where a functional group is a base, its pharmaceutically acceptable salt is in the scope of this invention.

In one embodiment, the preferred compounds are selected from the full-length peptide, A β (1-42, all-D), and its lower homologues consisting of A β (1-40, all-D), A β (1-35, all-D), and A β (1-28, all-D).

In another embodiment, the preferred compounds are selected from a group of short peptides, e.g., A β (1-7, all-D), A β (10-16, all-D), A β (16-21, all-D), A β (36-42, all-D). The peptides can be shortened further by removing one or more residues from either end or both ends.

The preferred compounds may also be all-D peptides derived from the peptides above by substitution of one or more residues in the naturally occurring sequence. In another embodiment, the preferred compounds are peptidomimetics of the above-said peptides.

In a further embodiment, the preferred compounds may be coupled with a carrier that will modulate the biodistribution, immunogenic property and the half-life of the compounds.

The following are exemplary compounds for preparing vaccines for preventing or treating Alzheimer's disease and other amyloid related diseases:

- 1 A β (1-42, all-D)
- 2 A β (1-40, all-D)
- 3 A β (1-35, all-D)
- 4 A β (1-28, all-D)
- 5 A β (1-7, all-D)
- 6 A β (10-16, all-D)
- 7 A β (16-21, all-D)
- 8 A β (36-42, all-D)
- 9 Lys-Ile-Val-Phe-Phe-Ala (all-D)
- 10 Lys-Lys-Leu-Val-Phe-Phe-Ala (all-D)

- 11 Lys-Phe-Val-Phe-Phe-Ala (all-D)
12 Ala-Phe-Phe-Val-Leu-Lys (all-D)
13 Lys-Leu-Val-Phe (all-D)
14 Lys-Ala-Val-Phe-Phe-Ala (all-D)
5 15 Lys-Leu-Val-Phe-Phe (all-D)
16 Lys-Val-Val-Phe-Phe-Ala (all-D)
17 Lys-Ile-Val-Phe-Phe-Ala-NH₂ (all-D)
18 Lys-Leu-Val-Phe-Phe-Ala-NH₂ (all-D)
19 Lys-Phe-Val-Phe-Phe-Ala-NH₂ (all-D)
10 20 Ala-Phe-Phe-Val-Leu-Lys-NH₂ (all-D)
21 Lys-Leu-Val-Phe-NH₂ (all-D)
22 Lys-Ala-Val-Phe-Phe-Ala-NH₂ (all-D)
23 Lys-Leu-Val-Phe-Phe-NH₂ (all-D)
24 Lys-Val-Val-Phe-Phe-Ala-NH₂ (all-D)
15 25 Lys-Leu-Val-Phe-Phe-Ala-Gln (all-D)
26 Lys-Leu-Val-Phe-Phe-Ala-Gln-NH₂ (all-D)
27 His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Gln (all-D)
28 Asp-Asp-Asp (all-D)
29 Lys-Val-Asp-Asp-Gln-Asp (all-D)
20 30 His-His-Gln-Lys (all-D)
31 Phe-Phe-NH-CH₂CH₂SO₃H (all-D)
32 Phe-Phe-NH-CH₂CH₂CH₂SO₃H (all-D)
33 Phe-Phe-NH-CH₂CH₂CH₂CH₂SO₃H (all-D)
34 Phe-Tyr-NH-CH₂CH₂SO₃H (all-D)
25 35 Phe-Tyr-NH-CH₂CH₂CH₂SO₃H (all-D)
36 Phe-Tyr-NH-CH₂CH₂CH₂CH₂SO₃H (all-D)
37 HO₃SCH₂CH₂-Phe-Phe (all-D)
38 HO₃SCH₂CH₂CH₂-Phe-Phe (all-D)
39 HO₃SCH₂CH₂CH₂CH₂-Phe-Phe (all-D)
30 40 HO₃SCH₂CH₂-Phe-Tyr (all-D)
41 HO₃SCH₂CH₂CH₂-Phe-Tyr (all-D)
42 HO₃SCH₂CH₂CH₂CH₂-Phe-Tyr (all-D)
43 HO₃SCH₂CH₂-Leu-Val-Phe-Phe-Ala (all-D)

- 44 $\text{HO}_3\text{SCH}_2\text{CH}_2\text{CH}_2\text{-Leu-Val-Phe-Phe-Ala (all-D)}$
- 45 $\text{HO}_3\text{SCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{-Leu-Val-Phe-Phe-Ala (all-D)}$
- 46 $\text{Leu-Val-Phe-Phe-Ala-NH-CH}_2\text{CH}_2\text{SO}_3\text{H (all-D)}$
- 47 $\text{Leu-Val-Phe-Phe-Ala-NH-CH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{H (all-D)}$
- 5 48 $\text{Leu-Val-Phe-Phe-Ala-NH-CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_3\text{H (all-D)}$.

The compounds listed above may be modified by removing or inserting one or more amino acid residues, or by substituting one or more amino acid residues with other amino acid or non-amino acid fragments.

The following are exemplary compounds derived from compound 18 (all-D KLVFFA-NH₂) by substituting one or two amino acid residue(s) with other amino acids.

- 49 $\text{Lys-Leu-Val-Trp-Phe-Ala-NH}_2\text{(all-D)}$
- 50 $\text{Lys-Leu-Val-Phe-Trp-Ala- NH}_2\text{ (all-D)}$
- 51 $\text{Lys-Leu-Val-Trp-Trp-Ala- NH}_2\text{ (all-D)}$
- 52 $\text{Lys-Leu-Val-Tyr-Phe-Ala- NH}_2\text{ (all-D)}$
- 15 53 $\text{Lys-Leu-Val-Phe-Tyr-Ala- NH}_2\text{ (all-D)}$
- 54 $\text{Lys-Leu-Val-Tyr-Tyr-Ala- NH}_2\text{ (all-D)}$
- 55 $\text{Lys-Leu-Val-Thi-Phe-Ala- NH}_2\text{ (all-D)}$
- 56 $\text{Lys-Leu-Val-Phe-Thi-Ala- NH}_2\text{ (all-D)}$
- 57 $\text{Lys-Leu-Val-Thi-Thi-Ala- NH}_2\text{ (all-D)}$
- 20 58 $\text{Lys-Leu-Val-Cha-Phe-Ala- NH}_2\text{ (all-D)}$
- 59 $\text{Lys-Leu-Val-Phe-Cha-Ala- NH}_2\text{ (all-D)}$
- 60 $\text{Lys-Leu-Val-Cha-Cha-Ala- NH}_2\text{ (all-D)}$
- 61 $\text{Lys-Leu-Val-Pgly-Phe-Ala- NH}_2\text{ (all-D)}$
- 62 $\text{Lys-Leu-Val-Phe-Pgly-Ala- NH}_2\text{ (all-D)}$
- 25 63 $\text{Lys-Leu-Val-Pgly-Pgly-Ala- NH}_2\text{ (all-D)}$.

For the above compounds, the terms Thi, Cha and Pgly are intended to mean thienylalanine, cyclohexylalanine and phenylglycine, respectively.

Rabbits were immunized with all-D or all-L KLVFFA. Results of the antibody titers obtained are shown in FIG. 7. As seen in FIG. 7, the vaccine of the present invention causes production of antibodies.

The present invention encompasses various types of immune responses triggered using the vaccine of the present invention, e.g., amyloid therapies using the vaccine approach.

In accordance with the present invention, there is also provided a vaccine which triggers a preferential TH-2 response or a TH-1 response, according to the type of immunization used. By inducing a TH-2 response, anti-inflammatory cytokine production such as IL-4, IL-10 and TGF- β , as well as the production of IgG 1 and IgG 2b antibody classes, are
5 favored. Such type of response would be preferred, as a major inflammatory response in the brain of the patients with AD would be avoided. On the other hand, with a preferred TH-1 response, a pro-inflammatory response with a production of inflammatory cytokines such as IL-1, IL-6, TNF and IFN gamma would be favored. This type of response would more likely trigger activation of the macrophage population. These macrophages would then phagocytose
10 any particulate deposits (such as plaques) via a complement-activated process as well as via antibody-mediated process. This approach would be beneficial to clear already organized senile plaques and prevent the formation of new fibrillary deposits.

Both approaches (i.e. TH-1 and TH-2) are of value. The antigen used could be the peptides which contain regions responsible for cellular adherence, i.e., region 10-16, regions
15 responsible for the GAG binding site, i.e., 13-16, regions responsible for the β sheet 16-21 or regions for 40-42. These peptides could be presented in such a way that either a preferential TH-1 or TH-2 response is obtained, depending on the type of adjuvant used, or depending on the route of administration of the vaccine. For example, a mucosal immunization via nasal administration is possible, since it is known that such a route of administration would favor a
20 TH-2 response.

The present invention will be more readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

25 An *in vitro* validation procedure to test the effectiveness of all-D peptide vaccines derived from fibrillogenic proteins was performed in rabbits or mice to demonstrate that antibodies can be raised against A β 16-21 (all-D) (see FIG. 7). The antibodies produced were tested to prove that they effectively prevent the fibrillogenesis of natural A β (1-40, all-L) *in vitro*. Standard assays for fibrillogenesis were used to evaluate activity, such as those based on
30 Thioflavine T, circular dichroism and solubility.

This approach could also be used to establish which areas of the A β peptide are most effective when used in the form of all-D peptides to prepare antifibrillogenic vaccines. One way this could be performed is as follows:

35 a) rabbits or mice are immunized with a series of overlapping all-D peptides generated from the A β (1-42) sequence, e.g., A β (1-6), A β (2-8), A β (4-10), etc.

- b) antisera are prepared from the immunized rabbits or mice.
- c) these antisera are tested to see which parts of the A β sequence produce antisera which most effectively prevents fibrillogenesis in the standard assays for fibrillogenesis mentioned above.

5

EXAMPLE II

Effect of Antibodies Against D- and L-A β (16-21) Peptide Vaccine on Fibrillogenesis

A validation procedure to test anti-fibrillogenic activity of antibodies raised against D- and L- A β (16-21) peptide was performed.

10 Rabbits were immunized with D- or L-A β (16-21) peptide. Antibodies raised were tested for their antifibrillogenic activities by ThT assay and by electron microscopy (EM).

Antibodies raised against the D- and L- forms of KLVFFA were capable of blocking the fibrillogenesis process as seen either by the Thioflavin T assay (ThT) (FIGs. 2 and 3) and by EM (FIGs. 4A to 4C). In the ThT assay, fibril formation is monitored by the increase in
15 fluorescence with time. As seen in the Figures, the antibodies were capable of inhibiting such an increase in fluorescence, proving that these antibodies were inhibiting fibrillogenesis.

As can be seen in these figures (FIGs. 2 to 4), antibodies raised against the D-peptide have a better anti-fibrillogenic activity than anti-L antibodies.

These results were also confirmed by EM (FIGs. 4A to 4C) where both anti-D and
20 anti-L KLVFFA peptide blocked the fibril formation when compared to control (FIG. 4A). Moreover, again the anti-D peptide has a greater anti-fibrillogenic activity (FIG. 4B) than the anti-L peptide (FIG. 4C). This goes along with the ThT assay where the decrease in fluorescence was greater with the anti-D peptide antibody than with the anti-L peptide antibody.

25

EXAMPLE III

Antibody Binding Assay

Brain sections were stained with antibodies raised against KLVFFA peptide (D and L forms). As seen in FIGs 5A to 5D and 6A to 6D, the antibodies were not capable of binding to aggregated (ThioS positive) A β . It can be seen from both sets of figures, which were stained
30 for both plaques (ThioS) and anti-peptides that the antibodies are recognizing A β at the surface of the cells but are not capable of binding to plaques. These results show that the anti-KLVFFA peptide antibody is recognizing the non-fibrillary A β but does not bind to aggregated A β . There was no difference between the anti-D and anti-L peptide antibodies in this assay.

These results clearly prove that the antibody recognizes only the non-aggregated form and blocks the fibrillogenesis. By having such activity, the vaccine of the present invention 1) prevents A β from organizing itself into a fibril and 2) prevents an inflammatory response being triggered by such an antibody binding to an insoluble form, since the antibody is not able to bind to aggregated A β .

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

CLAIMS

What is claimed is:

1. A method for preventing and/or treating an amyloid-related disease in a subject, comprising administering to a subject an antigenic amount of an all-D peptide which elicits production of antibodies against said all-D peptide, and elicit an immune response by said subject, therefore preventing fibrillogenesis and associated cellular toxicity, wherein said antibodies and/or immune cells interact with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof..
2. The method of claim 1, wherein said compound is a compound of Formula I:
$$R'-(P)-R'' \text{ (I),}$$

wherein
P is an all-D peptide interacting with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;
R' is an N-terminal substituent selected from the group consisting of:
hydrogen;
lower alkyl groups selected from the group consisting of acyclic or cyclic having 1 to 8 carbon atoms;
aromatic groups;
heterocyclic groups; and
acyl groups; and
R'' is a C-terminal substituent selected from the group consisting of hydroxy, alkoxy, aryloxy, unsubstituted or substituted amino groups.
3. The method of claim 2, wherein said alkyl or aryl group of R' and R'' is further substituted with halide; hydroxyl, alkoxyl, aryloxy, hydroxycarbonyl, alkoxycarbonyl, aryloxy carbonyl, carbamyl, unsubstituted or substituted amino, sulfo or alkyloxysulfonyl, phosphono or alkoxyphosphonyl groups.

4. The method of claim 2, wherein said compound further comprises an acid functional group, a pharmaceutically acceptable salt or ester form thereof; or a base functional group or pharmaceutically acceptable salt form thereof.

5

5. The method of claim 2, wherein said compound is selected from the group consisting of compounds 1 to 48.

10

6. The method of claim 5, wherein said compound is modified by removing or inserting one or more amino acid residues, or by substituting one or more amino acid residues with other amino acid or non-amino acid fragment.

15

7. The method of claim 6, wherein said compound is selected from the group consisting of compounds 49 to 63.

8. The method of claim 1, wherein said subject is a human being.

9. The method of claim 1, wherein said disease is Alzheimer's disease.

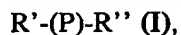
20

10. A vaccine for preventing and/or treating an amyloid-related disease in a subject, comprising an antibody raised against an antigenic amount of an all-D peptide which interacts with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof, wherein said antibody interacts with amyloid proteins and therefore prevents fibrillogenesis.

25

11. The vaccine of claim 10, wherein said compound is a compound of Formula I:

30



wherein

P is an all-D peptide interacting with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;

35

R' is an N-terminal substituent selected from the group consisting of:
hydrogen;
lower alkyl groups selected from the group consisting of acyclic or cyclic having
1 to 8 carbon atoms;
5 aromatic groups;
heterocyclic groups; and
acyl groups; and

R'' is a C-terminal substituent selected from the group consisting of hydroxy,
alkoxy, aryloxy, unsubstituted or substituted amino groups.

10

12. The vaccine of claim 10, wherein said alkyl or aryl group of R' and R'' are further substituted with halide; hydroxyl, alkoxyl, aryloxy, hydroxycarbonyl, alkoxycarbonyl, aryloxycarbonyl, carbamyl, unsubstituted or substituted amino, sulfo or alkoxysulfonyl, phosphono or alkoxyphosphonyl groups.

15

13. The vaccine of claim 11, wherein said compound further comprises an acid functional group, a pharmaceutically acceptable salt or ester form thereof; or a base functional group or pharmaceutically acceptable salt form thereof.

20

14. The vaccine of claim 10, wherein said compound is selected from the group consisting of compounds 1 to 48.

25

15. The vaccine of claim 11, wherein said compound is modified by removing or inserting one or more amino acid residues, or by substituting one or more amino acid residues with other amino acid or non-amino acid fragment.

16. The method of claim 15, wherein said compound is selected from the group consisting of compounds 49 to 63.

30

17. The vaccine of claim 10, wherein said subject is a human being.

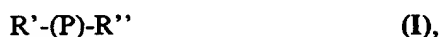
18. The vaccine of claim 10, wherein said disease is Alzheimer's disease.

35

19. Use of a vaccine as defined in claim 10, 11, 12, 13, 14, 15, 16, 17 or 18 for preventing and/or treating an amyloid-related disease.

20. Use of a vaccine as defined in claim 10, 11, 12, 13, 14, 15, 16, 17 or 18 for the manufacture of a medicament for preventing and/or treating an amyloid-related disease.
- 5 21. A method for preventing and/or treating an amyloid-related disease in a subject, comprising administering to said subject an antigenic amount of an all-D peptide which interacts with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof, wherein said compound elicits an immune response by said subject and therefore prevents fibrillogenesis.
- 10 22. The method of claim 21, wherein said compound is a compound of Formula I:
- 15
$$R'-(P)-R'' \quad (I),$$
- wherein
- P is an all-D peptide interacting with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;
- 20 R' is an N-terminal substituent selected from the group consisting of:
- hydrogen;
- lower alkyl groups selected from the group consisting of acyclic or cyclic having 1 to 8 carbon atoms;
- 25 aromatic groups;
- heterocyclic groups; and
- acyl groups; and
- R'' is a C-terminal substituent selected from the group consisting of hydroxy, alkoxy, aryloxy, unsubstituted or substituted amino groups.
- 30 23. The method of claim 22, wherein said alkyl or aryl group of R' and R'' are further substituted with halide; hydroxyl, alkoxyl, aryloxy, hydroxycarbonyl, alkoxycarbonyl, aryloxy carbonyl, carbamyl, unsubstituted or substituted amino, sulfo or alkyloxysulfonyl, phosphono or alkoxyphosphonyl groups.
- 35

24. The method of claim 22, wherein said compound further comprises an acid functional group, a pharmaceutically acceptable salt or ester form thereof; or a base functional group or a pharmaceutically acceptable salt form thereof.
- 5 25. The method of claim 22, wherein said compound is selected from the group consisting of compounds 1 to 48.
- 10 26. The method of claim 25, wherein said compound is modified by removing or inserting one or more amino acid residues, or by substituting one or more amino acid residues with other amino acid or non-amino acid fragment.
27. The method of claim 26, wherein said compound is selected from the group consisting of compounds 49 to 63.
- 15 28. The method of claim 21, wherein said subject is a human being.
29. The method of claim 21, wherein said disease is Alzheimer's disease.
- 20 30. A method for preventing and/or treating of an amyloid related disease in a subject, which comprises administering to the said subject an antigenic amount of a compound of Formula I:



wherein

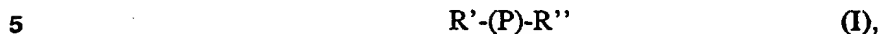
- 25 P is an all-D peptide interacting with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;
- 30 R' is an N-terminal substituent selected from the group consisting of:
hydrogen;
lower alkyl groups selected from the group consisting of acyclic or cyclic having 1 to 8 carbon atoms;
aromatic groups;
35 heterocyclic groups; and
acyl groups; and

R'' is a C-terminal substituent selected from the group consisting of hydroxy, alkoxy, aryloxy, unsubstituted or substituted amino groups.

- 5 31. The method of claim 30, wherein said compound elicits an immune response by said subject and therefore prevents fibrillogenesis.
- 10 32. The method of claim 30, wherein the alkyl or aryl group of R' and R'' are further substituted with halide; hydroxyl, alkoxyl, aryloxy, hydroxycarbonyl, alkoxycarbonyl, aryloxy carbonyl, carbamyl, unsubstituted or substituted amino, sulfo or alkyloxysulfonyl, phosphono or alkoxyphosphonyl groups.
- 15 33. The method of claim 32, wherein said compound has an acid functional group, a pharmaceutically acceptable salt or ester form thereof; or a base functional group or pharmaceutically acceptable salt thereof.
34. The method of claim 30, wherein said compound is selected from the group consisting of compounds 1 to 48.
- 20 35. The method of claim 34, wherein said compound is modified by removing or inserting one or more amino acid residues, or by substituting one or more amino acid residues with other amino acid or non-amino acid fragment.
- 25 36. The method of claim 35, wherein said compound is selected from the group consisting of compounds 49 to 63.
37. The method of claim 30, wherein said subject is a human being.
38. The method of claim 30, wherein said disease is Alzheimer's disease.
- 30 39. A vaccine for preventing and/or treating an amyloid-related disease in a subject, comprising an antigenic amount of an all-D peptide interacting with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, protein conjugates thereof, immunogenic derivative peptides thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof, wherein said
- 35

compound elicits an immune response by said subject and therefore prevents fibrillogenesis.

40. The vaccine of claim 39, wherein said compound is a compound of Formula I:



wherein

P is an all-D peptide interacting with at least one region of an amyloid protein selected from the group consisting of β sheet region and GAG-binding site region, A β (1-42, all-D), immunogenic fragments thereof, immunogenic derivatives thereof, protein conjugates thereof, immunogenic peptides thereof, and immunogenic peptidomimetics thereof;

R' is an N-terminal substituent selected from the group consisting of:

hydrogen;

lower alkyl groups selected from the group consisting of acyclic or cyclic having 1 to 8 carbon atoms;

aromatic groups;

heterocyclic groups; and

acyl groups; and

R'' is a C-terminal substituent selected from the group consisting of hydroxy, alkoxy, aryloxy, unsubstituted or substituted amino groups.

41. The vaccine of claim 40, wherein said alkyl or aryl group of R' and R'' is further substituted with halide; hydroxyl, alkoxyl, aryloxy, hydroxycarbonyl, alkoxycarbonyl, aryloxy carbonyl, carbamyl, unsubstituted or substituted amino, sulfo or alkyloxysulfonyl, phosphono or alkoxyphosphonyl groups.

42. The vaccine of claim 40, wherein said compound has an acid functional group, a pharmaceutically acceptable salt or ester form thereof; or said compound has a base functional group or pharmaceutically acceptable salt form thereof.

43. The vaccine of claim 39, wherein said compound is selected from the group consisting of compounds 1 to 48.

44. The vaccine of claim 40, wherein said compound is modified by removing or inserting one or more amino acid residues, or by substituting one or more amino acid residues with other amino acid or non-amino acid fragment.
- 5 45. The method of claim 44, wherein said compound is selected from the group consisting of compounds 49 to 63.
46. The vaccine of claim 39, wherein said subject is a human being.
- 10 47. The vaccine of claim 39, wherein said disease is Alzheimer's disease.
48. Use of a vaccine as defined in claim 39, 40, 41, 42, 43, 44, 45, 46 or 47 for preventing and/or treating an amyloid-related disease.
- 15 49. Use of a vaccine as defined in claim 39, 40, 41, 42, 43, 44, 45, 46 or 47 for the manufacture of a medicament for preventing and/or treating an amyloid-related disease.

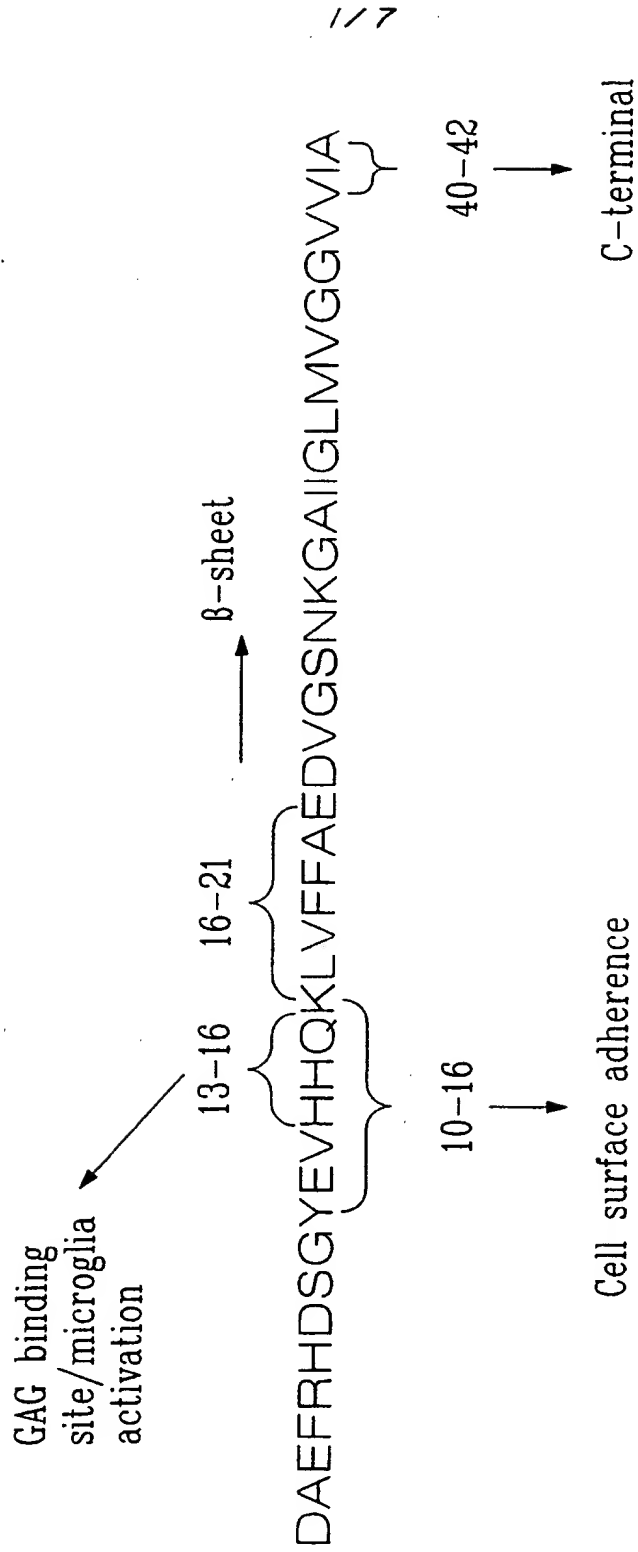
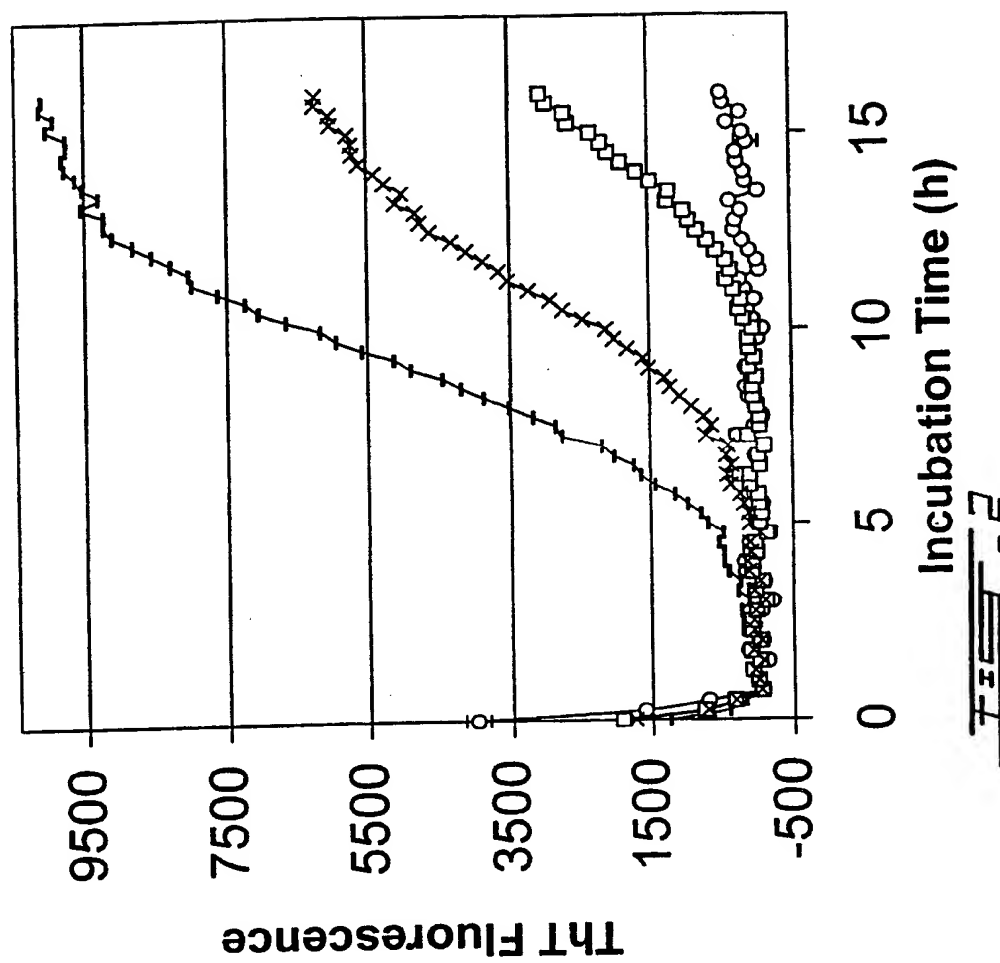


FIG. 1

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○ Anti [D]-KLVFFA

— No antibodies

□ Anti [L]-KLVFFA

* Control antibody

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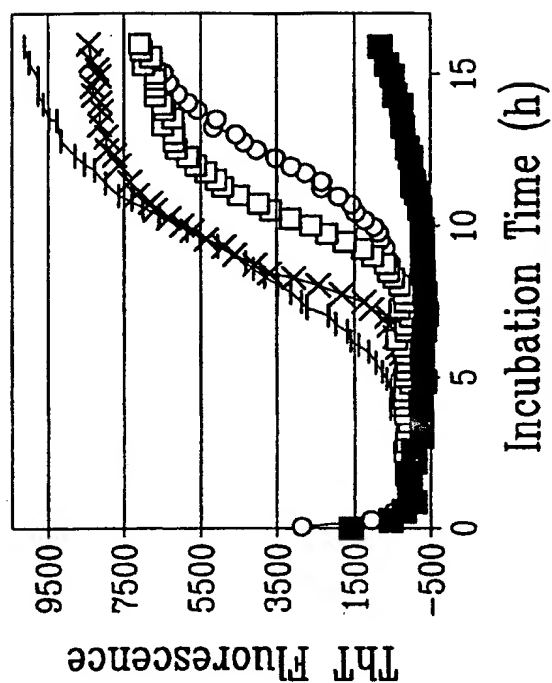
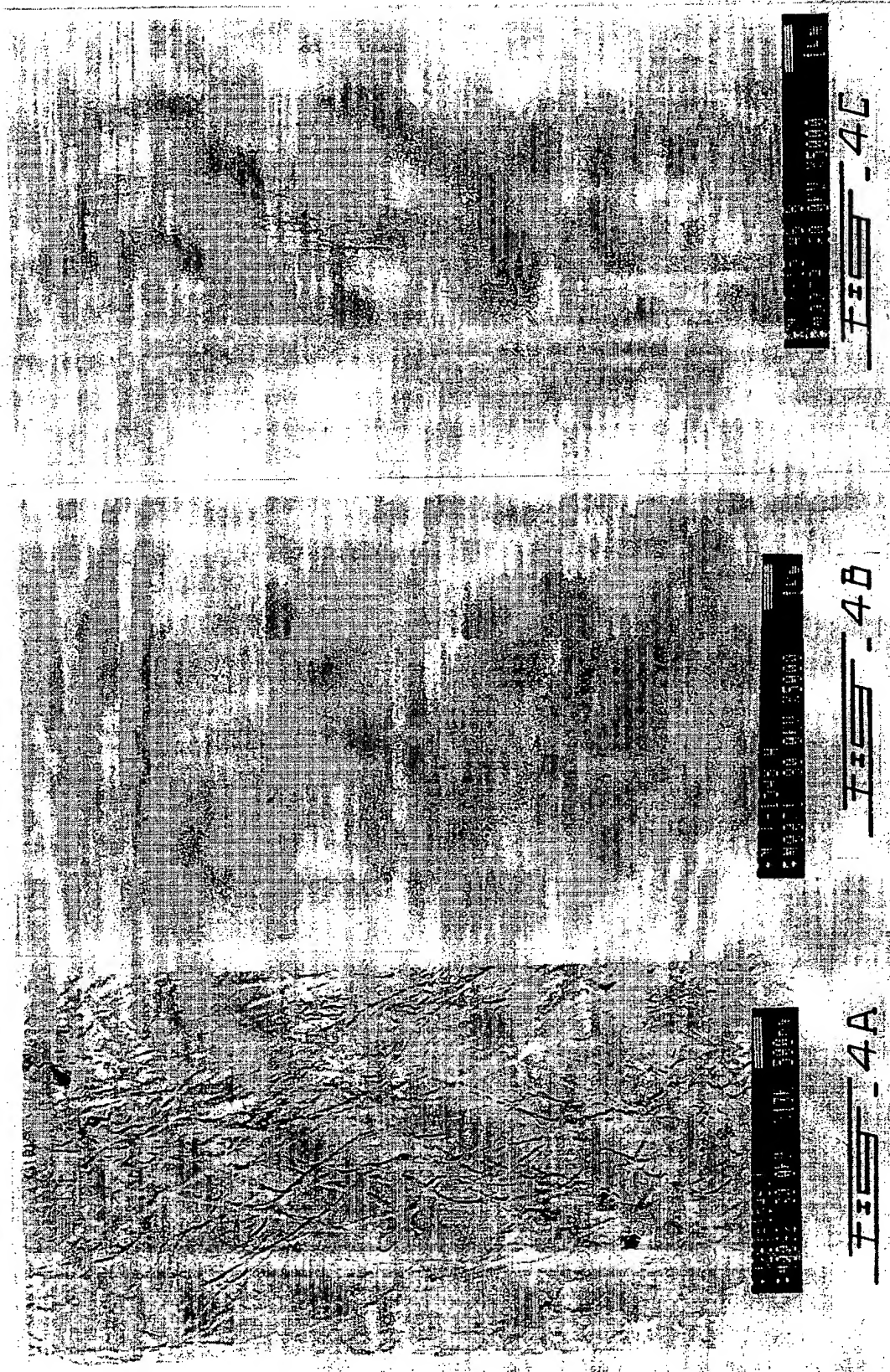


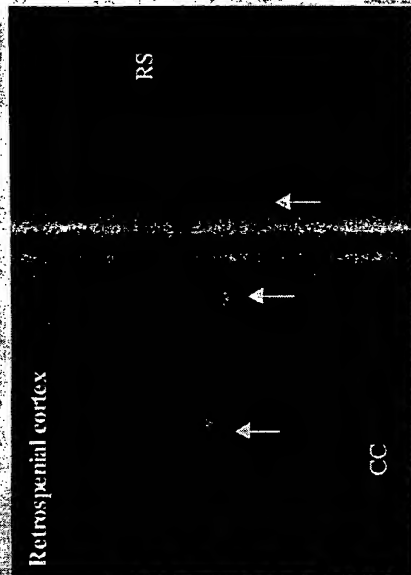
Figure 3

- Anti [D]-KLVFFA
- No antibodies
- × Control antibody
- Anti [L]-KLVFFA
- Anti [D]-KLVFFA binding [L]-KLVFFA



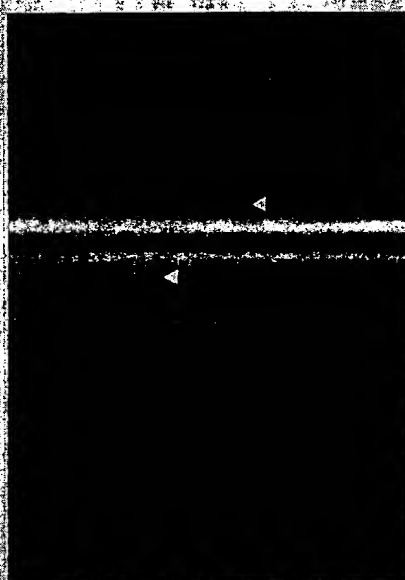
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HISTOCHEMISTRY FOR THIOFLAVIN S



THIOFLAVIN S

HISTOCHEMISTRY FOR THIOFLAVIN S



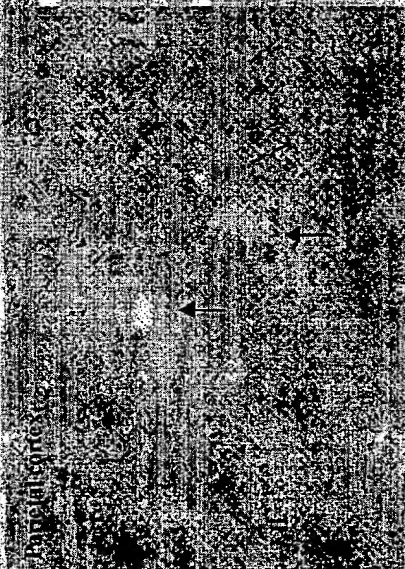
THIOFLAVIN S

IMMUNOHISTOCHEMISTRY FOR A 151



A 151

IMMUNOHISTOCHEMISTRY FOR A 151



A 151

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HISTOCHEMISTRY FOR THIOFLAVIN S

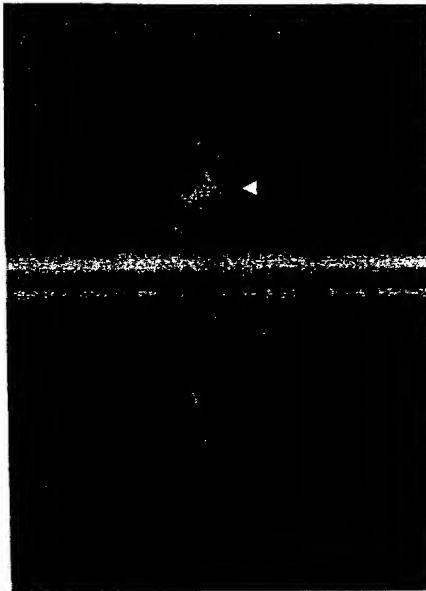


Fig. 6A

HISTOCHEMISTRY FOR THIOFLAVIN S

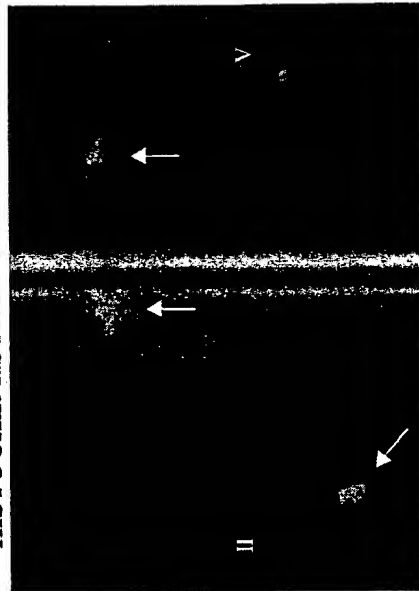


Fig. 6B

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Fig. 6A

IMMUNOHISTOCHEMISTRY FOR C151

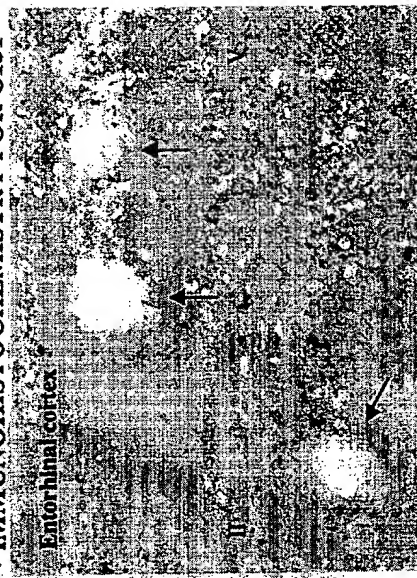
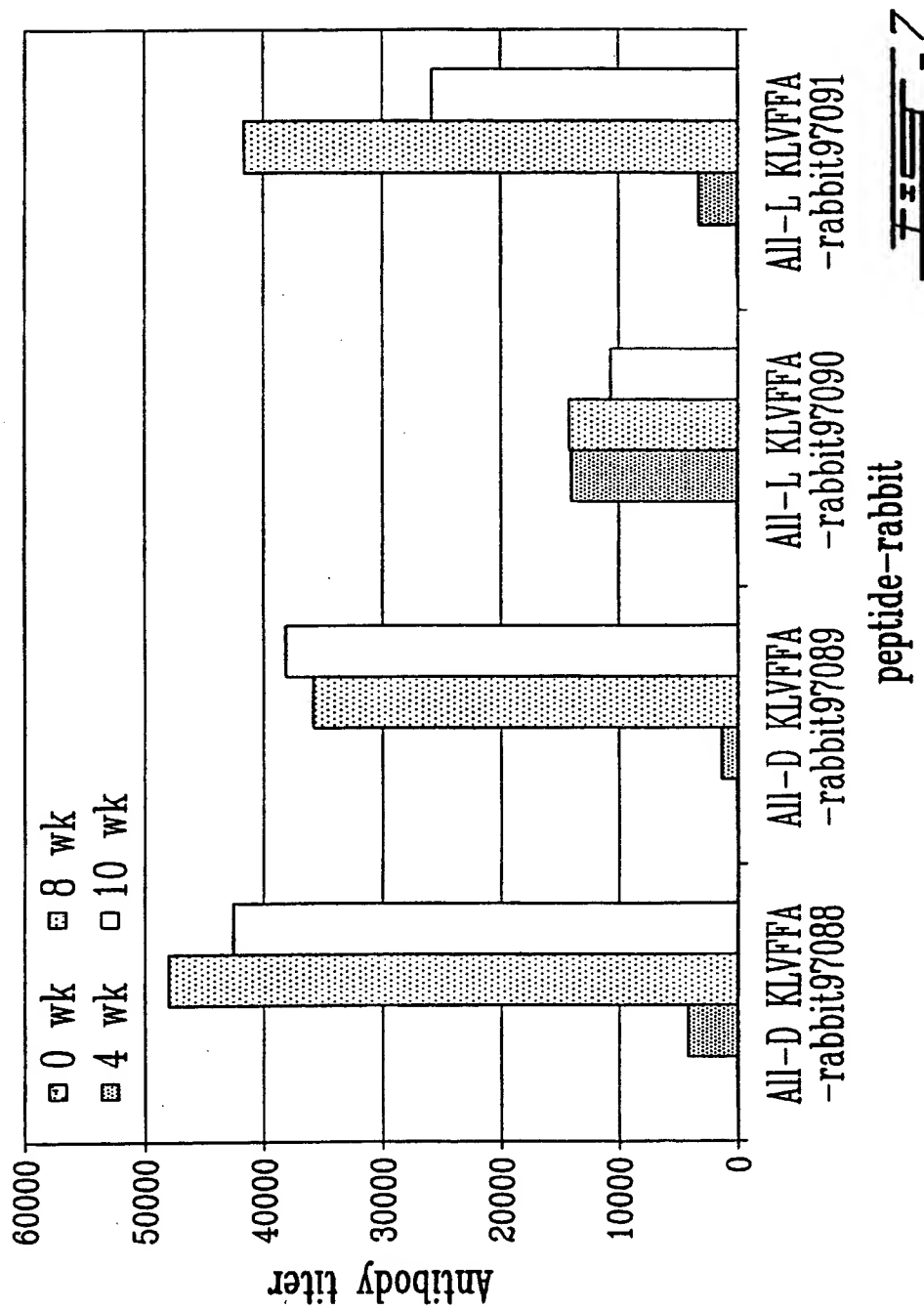


Fig. 6B

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(19) World Intellectual Property Organization
International Bureau



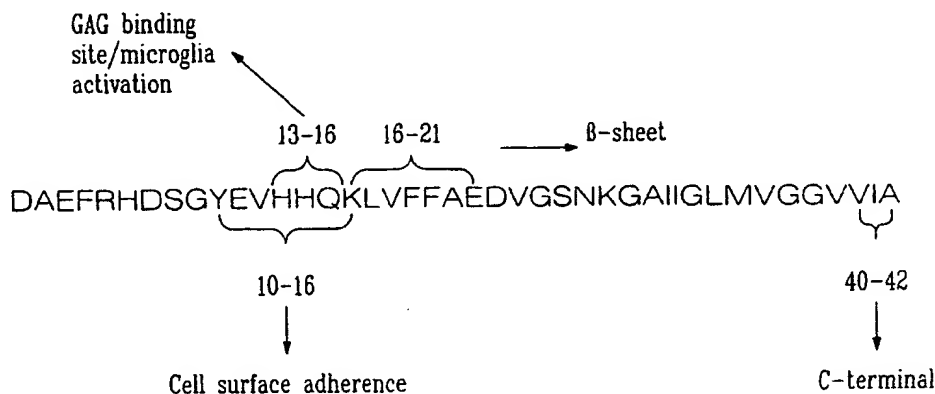
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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
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6 December 2001
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **VACCINE FOR THE PREVENTION AND TREATMENT OF ALZHEIMER'S AND AMYLOID RELATED DISEASES**



(57) Abstract: The present invention relates to a stereochemically based "non-self" antigen vaccine for the prevention and/or treatment of Alzheimer's and other amyloid related diseases. The present invention provides a vaccine for the prevention and treatment of Alzheimer's and other amyloid related diseases, which overcomes the drawbacks associated with using naturally occurring peptides, proteins or immunogens.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/01413

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 A61K39/00 A61K39/395 A61P25/28		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) MEDLINE, LIFESCIENCES, EMBASE, CHEM ABS Data, SCISEARCH, BIOSIS, EPO-Internal		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 08868 A (PRAECIS PHARM INC) 5 March 1998 (1998-03-05) page 3, line 19-29 page 4, line 7-21 page 25, line 26 claims	21-38
P, X	WO 00 68263 A (NEUROCHEM INC ;GUPTA AJAY (CA); CHALIFOUR ROBERT (CA); GERVAIS FRA) 16 November 2000 (2000-11-16) page 5, line 14-31 page 6, line 20 -page 7, line 31 claims	1-9, 21-38
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<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *8* document member of the same patent family		
Date of the actual completion of the international search 22 May 2001		Date of mailing of the international search report 06/06/2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016		Authorized officer Covone, M

INTERNATIONAL SEARCH REPORT

International Application No

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